

## RELATIVE SUSCEPTIBILITY OF *Helicoverpa armigera* (HÜBNER) AND *Spodoptera litura* FABRICIUS POPULATION TO BOLLGARD II COTTON

<sup>1\*</sup>Datchina Murthy K, Manoharan T and Divya S

Department of Agricultural Entomology, TNAU, Coimbatore, Tamil Nadu, India, 641 003

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### ABSTRACT

Transgenic cotton, expressing Cry1Ac and Cry2Ab insecticidal  $\delta$ -endotoxin protein of *Bacillus thuringiensis* Berliner often referred as Bt cotton, is widely grown in many countries. Bt cotton with a single *cry1Ac* gene and stacked also with *cry2Ab* gene provided satisfactory protection against major lepidopteran pests. A survey has been conducted in different cotton growing areas of Tamil Nadu to study the relative susceptibility of key lepidopteran pests viz., *Helicoverpa armigera* (Hübner) and *Spodoptera litura* Fab. to Bt cotton. Bioassay studies indicated that, *H. armigera* population collected from Madurai region recorded a maximum susceptibility of 96.67 per cent and Coimbatore population recorded a minimum susceptibility of 83.33 per cent to bollgard II (RCH 2 Bt). In the case of *S. litura*, the highest susceptibility was recorded from the population collected from Madurai region (70.00%) and lowest from Salem region (53.33).

**Keywords:** Susceptibility, Baseline susceptibility, *Helicoverpa armigera*, *Spodoptera litura*, Bt Cotton and Resistance.

### INTRODUCTION

Cotton (*Gossypium* Spp. L.) is an important fiber crop of global significance widely cultivated in tropical and sub-tropical regions of more than seventy countries. Owing to their damage potential, insect pests of cotton are the most important constraint in production. The green bollworm, *Helicoverpa armigera* Hüb is an important key pest which has developed resistance against all the group insecticides used for its control (Armes *et al.*, 1996). Leaf eating caterpillar, *Spodoptera litura* Fab is one of major polyphagous pest (Qin and Ye, 2007). In the past decades, *S. litura* has developed high levels of resistance to insecticides (Sang *et al.*, 2013). In addition, efficacy of *S. litura* has been questioned, raising concerns about low susceptibility or development of resistance against Bt (Shu Yinghua *et al.*, 2017).

Among the insecticidal proteins produced by *Bacillus thuringiensis* Berliner Cry1Ac is the most toxic to *H. armigera* which has been utilized in transgenic Bt cotton and many commercial Bt cotton hybrids have been widely grown all over India. The transgenic cotton showed great resistance against *H. armigera* both under laboratory and field conditions (Venugopal *et al.*, 2002). The area under Bt

cotton in India has been increased from 12 per cent in 2002 to nearly 95 per cent in 2022 (Saikat *et al.*, 2022). Laboratory experiments conducted in India (Gujar *et al.*, 2001), Australia (Akhurst *et al.*, 2003) and China (Liang *et al.*, 2000) have shown that *H. armigera* is capable of developing resistance to Cry1Ac toxin. Since, the Bt cotton expresses the Cry toxin in all parts of the plant throughout the growth period and wide-spread commercial deployment of Bt cotton, the pest would thus be exposed to a continuous selection pressure, thereby causing resistance in field populations.

The variation in expression of the protein among different plant parts or avoidance by the target insects to feed on transgenic crop having significant importance in efficacy (Adamczyk *et al.*, 2001). Also, *H. armigera* larvae have frequent movement, potentially within and between plants, imply that larvae may be able to detect and avoid structures with high expression of cry toxins and selectively feed on tissues with lower expression (Luong *et al.*, 2022). Information on resistance monitoring in Bt cotton helps immensely in devising proactive resistance management strategies that can retard the rate of resistance development. Keeping this in view, this study was conducted to establish

\*Corresponding Author: Datchina Murthy K, Department of Agricultural Entomology, TNAU, Coimbatore 641 003, Tamil Nadu, India Email: datchinas@gmail.com.

the relative susceptibility of the pests to Bt cotton which could provide a baseline data for resistance monitoring.

## MATERIALS AND METHODS

### Mass culturing of host insects

Laboratory experiments were carried out during 2020-21 to study the susceptibility level of third instar larvae of *H. armigera* and *S. litura* collected from major cotton growing areas of Tamil Nadu against Bollgard II Bt (RCH 2 Bt). Grown up caterpillars of *H. armigera* and *S. litura* collected from cotton growing areas of Coimbatore, Salem, Dharmapuri, Perambalur and Madurai regions during cropping season of 2020-2021 and reared till pupation on a chickpea based semi synthetic diet as described by Sathiah (2001). The different instars of the F<sub>1</sub> generation of field collected populations were utilized for bioassay studies.

### Culturing of Host plants

Bollgard II Bt cotton (RCH 2 Bt) and non Bt cotton (RCH 2) hybrids were obtained from Rasi Seeds (P) Ltd, Tamil Nadu and raised in micro plots. The host plants were maintained pest free condition.

### Feeding Bioassay

Bioassays were conducted by leaf disk method as described by Kranthi *et al.* (1999) with 75 days old Bt and non Bt cotton. The fully opened fourth leaves from two to three

nodes below the terminal end were collected and the petioles of leaves were inserted into 2 per cent agar in a small Petri plate to maintain the turgidity. Third instar larvae of *H. armigera* and *S. litura* were released separately for each Petri plates for each location. Each treatment was replicated thrice and at least 10 larvae formed one experimental unit. Mortality of the larvae was recorded every 24 hours for 7 days. The larvae were marked dead when they did not move when prodded. All the experiments were carried out in a room with light regime of 14: 10 (L: D), 27±1 °C and 60 per cent relative humidity.

## RESULTS AND DISCUSSION

Variation in the susceptibility of *H. armigera* was noticed from the population collected in different locations of Tamil Nadu against Bollgard II Bt cotton (RCH 2 Bt) (Table 1). On 120 hours after treatment (HAT), highest per cent mortality of 80.00 was recorded in Madurai and Dharmapuri population, which was followed by 66.67, 53.33 and 50.00 per cent in Perambalur, Salem and Coimbatore populations respectively. At 168 HAT, the *H. armigera* population collected from Madurai region recorded the highest mortality of 96.67 per cent followed by 93.33 per cent from the population collected from Dharmapuri to BG II Bt cotton. Perambalur, Coimbatore, and Salem populations recorded 86.67, 83.33 and 80.00 per cent mortality respectively and were on par with each other. In non Bt cotton, the mortality was ranged from 3.33 to 10.00 per cent at 168 HAT.

**Table1.** Susceptibility of III instar *H. armigera* larvae collected from different parts of Tamil Nadu to BG II Bt cotton.

Location	Cumulative Mean per cent mortality*							
	Bt leaf				Non Bt leaf			
	24 HAT	72 HAT	120 HAT	168 HAT	24 HAT	72HAT	120 HAT	168 HAT
Coimbatore	3.33 (10.52)	26.67 (31.09)	50.00 <sup>b</sup> (45.00)	83.33 <sup>bc</sup> (65.91)	0.00 (0.00)	3.33 (10.52)	3.33 (10.52)	3.33 (10.52)
Salem	10.00 (18.43)	36.67 (37.27)	53.33 <sup>b</sup> (46.91)	80.00 <sup>c</sup> (63.43)	3.33 (10.52)	3.33 (10.52)	6.67 (14.96)	6.67 (14.96)
Dharmapuri	3.33 (10.52)	30.00 (33.21)	80.00 <sup>a</sup> (63.43)	93.33 <sup>ab</sup> (75.03)	0.00 (0.00)	3.33 (10.52)	6.67 (14.96)	10.00 (18.43)
Perambalur	3.33 (10.52)	26.67 (31.09)	66.67 <sup>ab</sup> (54.74)	86.67 <sup>bc</sup> (68.58)	0.00 (0.00)	3.33 (10.52)	6.67 (14.96)	6.67 (14.96)
Madurai	3.33 (10.52)	30.00 (33.21)	80.00 <sup>a</sup> (63.43)	96.67 <sup>a</sup> (79.48)	0.00 (0.00)	3.33 (10.52)	6.67 (14.96)	6.67 (14.96)
SEd	9.2246	4.0278	5.6700	6.0068	3.8854	8.6880	8.6880	7.7708
CD (P=0.05)	NS	NS	12.633	13.384	NS	NS	NS	NS

HAT: Hours after Treatment, \*mean of three replications, NS: Non significant, Figures in the parenthesis are arcsine transformed values. Means followed by same letter(s) in the same column are not significantly different (P=0.05)

**Table 2.** Susceptibility of third instar *S. litura* collected from different parts of Tamil Nadu to BG II Bt cotton.

Location	Cumulative Mean per cent mortality*							
	Bt leaf				Non Bt leaf			
	24 HAT	72 HAT	120 HAT	168 HAT	24 HAT	72 HAT	120 HAT	168 HAT
Coimbatore	3.33 (10.52)	16.67 (24.09)	50.00 (45.00)	56.67 <sup>bc</sup> (48.83)	0.00	3.33 (10.52)	3.33 (10.52)	3.33 (10.52)
Salem	0.00 (0.00)	20.00 (26.57)	43.33 (41.17)	53.33 <sup>c</sup> (46.91)	0.00	6.67 (14.96)	6.67 (14.96)	6.67 (14.96)
Dharmapuri	3.33 (10.52)	23.33 (28.88)	50.00 (45.00)	60.00 <sup>bc</sup> (50.77)	0.00	3.33 (10.52)	3.33 (10.52)	3.33 (10.52)
Perambalur	0.00 (0.00)	16.67 (24.09)	46.67 (43.09)	63.33 <sup>ab</sup> (52.73)	0.00	3.33 (10.52)	6.67 (14.96)	6.67 (14.96)
Madurai	0.00 (0.00)	16.67 (24.09)	43.33 (41.17)	70.00 <sup>a</sup> (56.79)	0.00	3.33 (10.52)	3.33 (10.52)	3.33 (10.52)
SEd	5.4948	5.0843	4.2138	2.1378	0.0000	8.6880	8.6880	8.6880
CD (P=0.05)	NS	NS	NS	4.7633	NS	NS	NS	NS

HAT: Hours after Treatment, \*Mean of three replications, NS: Non significant. Figures in the parenthesis are arcsine transformed values. Means followed by same letter(s) in the same column are not significantly different (P=0.05)

In initial period of observation, there was no significant difference in the mortality of *S. litura* was observed among the populations of different regions. At 168 HAT, the highest susceptibility of *S. litura* was noticed in Madurai (70.00%) and Perambalur (63.33%) populations which was followed by Dharmapuri (60.00), Coimbatore (56.67) and Salem (53.33) and were on par with each other. The non Bt cotton recorded the minimum per cent mortality and it ranged from 3.33 to 6.67 at 168 HAT (Table 2).

The results of the present study indicate the minimum variation in susceptibility levels of *H. armigera* and *S. litura* to transgenic Bt cotton (RCH 2 Bt) across the Tamil Nadu. Gujar *et al.* (2001) conducted the experiment with more 15 *H. armigera* population collected from across India and reported 10 fold variations in the susceptibility among the population. Fakrudin *et al.* (2003) also observed seven fold variations in susceptibility of *H. armigera* to Cry1Ac insecticidal protein in India). The geographic variation in susceptibility of *H. armigera* to Bt insecticidal protein was reported by Gujar *et al.* (2001), who found that the populations of Delhi, Raichur and Bangalore were least susceptible whereas Hyderabad and Madurai populations were highly susceptible to Bt protein. Of the several field strains tested from various locations in the country, *H. armigera* from Akola, Rangareddy and Guntur were found to be tolerant to Cry1Ac toxin (67 fold tolerance); and Prakasham strain exhibited the highest tolerance to Cry 1 Ac which was significantly higher than all strains except the Coimbatore strain (Kranthi *et al.*, 2000). The susceptibility of *H. armigera* to Cry1Ac protein varied geographically in India, China and USA (Kranthi *et al.*, 2009). In the present observation, the susceptibility level during 2020-21 ranged from 80 to 96.67 per cent which is slightly less when compared to earlier observation by Jayaprakash *et al.* (2013) who reported 86.67 to 100 per cent susceptibility of *H. armigera* to bollgard II Bt cotton from the populations collected in different areas of Tamil

Nadu during 2009. This necessitating a need to develop strategies to be implemented immediately to delay any possible future resistance development.

## CONCLUSION

The variation in the baseline susceptibility data is helps immensely in devising proactive resistance management strategies that can delay the rate of resistance development. The development of resistance to Bt toxin can be quite distinct, depending upon the species, selection pressure or geographical region.

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## REFERENCES

- Adamczyk, J.J., Hardee, D.D., Adams, L.C, Sumerford, D V. (2001). Correlating differences in larval survival and development of bollworm (Lepidoptera: Noctuidae) and fall armyworm (Lepidoptera: Noctuidae) to differential expression of Cry1Ac  $\delta$ -endotoxin in various plant parts among commercial cultivars of transgenic *Bacillus thuringiensis* cotton. *Journal of Economic Entomology*, 94, 284 -290.
- Akhurst, R. J., James, W.L., Bird, L.J. and Beard, C. (2003). Resistance to the Cry1Ac delta endotoxin of *Bacillus thuringiensis* in the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Journal of Economic Entomology*, 96, 1290-1299.

- Armes, N.J., Jadhav, D. R. and Desouza, K.R. (1996). A survey of insecticide resistance in *Heliothis armigera* in the Indian subcontinent. *Bulletin of Entomological Research*, 86, 499-514.
- Fakrudin, B., Prakash, S.H., Krishnareddy, K.B. and Patil B.V. (2003). Baseline resistance to Cry1Ac protein in geographic populations of *Helicoverpa armigera* in south Indian cotton ecosystem. *Current Science*, 80, 1304-1307.
- Gujar, G.J., Archana Kumari, Vinay Kalia and Chandrashekar, K. (2001). Special and temporal variation in susceptibility of the American bollworm, *Helicoverpa armigera* (Hübner) to *Bacillus thuringiensis* var. *kurstaki* in India. *Current Science*, 78, 995-1001.
- Jayaprakash, S.A., Mohan, S. and Kannan, M. (2013). Temporal and spatial variation in susceptibility of *Helicoverpa armigera* (Hübner) populations to Bollgard II Bt cotton in Tamil Nadu, India. *Tropical Agricultural Research*, 24(3), 249-257.
- Kranthi, K.R., Kranthi, S., Ali, S. and Banerjee, S.K. (2000). Resistance to Cry1Ac  $\delta$ -endotoxin of *Bacillus thuringiensis* in a laboratory selected strain of *Helicoverpa armigera* (Hübner). *Current Science*, 78 (8), 1001-1004.
- Kranthi, S., Dhawad, S., Naidu, C.S., Bharose, A., Chaudhary, A., Sangode, V., Nehare, S.K., Bajaj, S.R. and Kranthi, K.R. (2009). Susceptibility of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) to the *Bacillus thuringiensis* toxin Cry2Ab before and after the introduction of Bollard II. *Crop Protection*, 28, 371-375.
- Kranthi, S., Kranthi, K. R. and Lavhe, N. V. (1999). Baseline toxicity of Cry1Ac toxins to the spotted bollworms *Earias vitella* F. *Crop Protection*, 18,551-555.
- Liang, G., Tan, W. and Guo, Y. (2000). Study on screening and mode of inheritance to Bt transgenic cotton in *H. armigera*. *Acta Entomologica Sinica*, 43, 57-62.
- Luong, T.T.A., Downes, S.J., Perkins L.E. and Zalucki M P. (2022). Drop-off behaviour of Bt-resistant and Bt-susceptible *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) larvae on Bt-cotton and non-Bt cotton plants. *Bulletin of Entomological Research*, 112, 604-612.
- Pilar Munoz, Carmen Lopez, Marian Moralejo, Meritxell Perez-Hedo and Matilde Eizaguirre. (2014) Response of Last Instar *Helicoverpa armigera* Larvae to Bt Toxin Ingestion: Changes in the Development and in the CYP6AE14, CYP6B2 and CYP9A12 *Gene Expression*. PLOS. <https://doi.org/10.1371/journal>.
- Qin, H. G., & Ye, Z. H. (2007). Occurrence and management of the common cutworm, *Spodoptera litura* in China. Beijing, China: Agricultural Science and Technology Press.
- Saikat, M., Padhy, C. and Sai Sruti, S. (2022). Analyzing the adoption of Bt cotton in India. *Asian Journal of Agricultural Extension, Economics & Sociology*, 40(12), 318-323.
- Sang, S., Wang, Z., Qi, J. W., Shu, B. S. and Zhong, G. H. (2013). Research progress on pesticide resistance of *Spodoptera litura*. *Journal of Environmental Entomology*, 35, 808-814.
- Sathiah, N. (2001). Studies on improving the production and formulation of the nuclear polyhedrosis virus of the cotton bollworm, *Helicoverpa armigera* (Hubner). Ph.D, Thesis, Tamil Nadu Agricultural University, Coimbatore, India, 276p.
- Shu Yinghua, Du Yan, Chen Jin, Wei Jiayi and Wang Jianwu. (2017). Responses of the cutworm *Spodoptera litura* (Lepidoptera: Noctuidae) to two Bt corn hybrids expressing Cry1Ab. *Nature*, 7. <https://doi.org/10.1038/srep41577>
- Venugopal, K., Ramasami, M. and Thigarajan, C.P. (2002). Risk assessment and its management in Bt cotton in India. In: Proceedings of National Seminar on Bt cotton Scenario with Special Reference to India, USA Dharwad, 23 may, pp.70-84.